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Original Article

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Nodular glomerulosclerosis with anti-glomerular basement membrane-like glomerulonephritis; a distinct pattern of kidney injury observed in smokers

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Abstract

Background. Cigarette smoking has recently been recognized as a risk factor for developing nodular glomerulosclerosis and has also been frequently encountered in patients with anti-glomerular basement membrane (anti-GBM) disease. However, the concurrent presence of both patterns of glomerular injury has not been previously reported.

Material and methods. In this article, we describe three patients with non-diabetic nodular glomerulosclerosis, anti-GBM-like glomerulonephritis (GN) and a history of heavy smoking.

Results. Our cohort included three patients, of which two were men (53 and 77 years old) and one a 28-year-old woman. None of the patients had a history of diabetes mellitus but all of them were heavy smokers who presented with renal insufficiency and proteinuria. Nodular glomerulosclerosis and occasional small, non-circumferential crescents in different stages of development were found on kidney biopsy. Immunofluorescence microscopy studies showed intense linear IgG staining along the glomerular basement membranes in the absence of granular immune-type deposits. Electron microscopy evaluation revealed prominent endothelial cell injury without detectable electron-dense deposits. One patient was dialysis-dependent a few months post-biopsy while the other two patients maintained their kidney function 18 and 24 months post-biopsy but without a significant improvement of serum creatinine.

Conclusions. The combination of nodular glomerulosclerosis and anti-GBM-like GN appears to be a distinct pattern of injury observed in a small subset of heavy smokers. Although this pattern of glomerular injury might be less aggressive than the typical anti-GBM GN, it does not appear to carry a favorable prognosis.

Keywords: anti-glomerular basement membrane glomerulonephritis; endothelial cell injury; nodular glomerulosclerosis; smoking

Introduction

Cigarette smoking has been recognized as a risk factor for development and progression of kidney damage [1, 2]. A study by Markowitz *et al.* [3] has shown that the vast majority of patients with 'idiopathic nodular glomerulosclerosis' (ING) were smokers. This association was soon confirmed by others [4–6]. Several studies have also proposed a link between cigarette smoking and anti-glomerular basement membrane (anti-GBM) disease. This concept was based on the fact that a high proportion of patients with Goodpasture syndrome were active smokers [7–9]. Despite the aforementioned independent association of smoking with nodular glomerulosclerosis and anti-GBM disease, no studies have previously documented the concurrent detection of both glomerular lesions in smoker patients. Here, we present the first report of three patients with non-diabetic nodular glomerulosclerosis and anti-GBM-like glomerulonephritis (GN) on a kidney biopsy.

Material and methods

The study was performed under the approval of the Brigham's and Women's Institutional Review Board (IRB# 2011P002692). Using our electronic reporting system, we found a combination of nodular glomerulosclerosis and features of anti-GBM-GN in three patients with kidney biopsies during past 7 years (3 of 7675 kidney biopsies; 0.03% of all kidney biopsies).

All three biopsies were performed at outside institutions. Kidney tissues from patients 2 and 3 were submitted to the Brigham and Women's Hospital (Boston, MA, USA)

for processing and diagnosis while the specimen from patient 1 was processed at the outside institution and the light microscopy slides, immunofluorescence microscopy images, and electron microscopy grids were submitted to our institution for consultation.

Standard processing of renal biopsies for light, immunofluorescence and electron microscopy was performed for each sample. For light microscopy evaluation, sections were stained with hematoxylin and eosin (H&E), periodic acid-Schiff (PAS), Jones' methenamine silver, and trichrome stains. For immunofluorescence microscopy assessment, the frozen sections were stained with antibodies specific for the heavy chains of IgG, IgM, and IgA, C3 and C1q complement components, kappa and lambda light chains, fibrin, and albumin. IgG subtyping with antibodies specific for heavy chains of IgG1, IgG2, IgG3 and IgG4 was also performed by the immunofluorescence technique for all three biopsies; for specimens from patients 1 and 2, IgG subtyping was performed and interpreted at the Mayo Clinic Laboratories (Rochester MN). For patient 3, IgG subtyping was performed and interpreted at the Brigham and Women's Hospital.

Results

Clinical and demographic features

This small cohort of patients included two men (53 and 77 years old) and one 28-year-old woman (Table 1). Two patients were Caucasian and one patient was Hispanic. None of the patients had a clinical diagnosis of diabetes mellitus but all of them were heavy smokers with a mean cumulative intake of 42 pack/year. Patient 3 with the lowest smoking cumulative intake (14 pack/year) is a 28 year-old female who has been an active heavy smoker for the last 8 years prior to this presentation with intermittent use of amphetamine. Noteworthy, none of the patients had a long history of hypertension but all of them developed a relatively recent increase in blood pressure (up to 3 months prior to biopsy). The patients did not present with a significant respiratory involvement such as pulmonary hemorrhage and only one patient had an isolated episode of mild hemoptysis. The three patients had mild lower extremity edema, microscopic or gross hematuria, and elevated serum creatinine values [mean 301 µmol/L (3.4 mg/dL)]. Protein was always detected in the urine and the amount of proteinuria ranged between 1 and 6.4 g/24 h. Laboratories revealed undetectable anti-neutrophilic cytoplasmic antibodies (ANCA), normal serum complement levels and non-reactive hepatitis B and C panels in all three patients. When performed, serum protein electrophoreses were negative (Table 1). ANA was negative in two patients while it was detectable in low titer in one patient (patient 2), who had negative anti-double-stranded DNA (ds-DNA) antibodies (Table 1). Urine culture was performed in one patient and was negative. Anti-GBM antibodies were always negative by serum ELISA and positive in 1 of 2 patients by Western blot (Table 1).

Histology

Tissue sampling for light microscopy included a mean of 28 glomeruli (range: 21–42 glomeruli). Globally sclerosed glomeruli were detected in all three samples (mean: 28% of total glomeruli) (Table 2). All renal biopsies revealed diffuse mesangial matrix expansion with nodule formation (Figure 1A–D) and foci of mesangiolysis (Figure 1A and B). The glomerular capillaries showed endothelial injury with occasional microaneurysmal dilatation (Figure 1A). The glomerular basement membranes also showed mild

Table 1. Demographic and clinical features

	Patient 1	Patient 2	Patient 3
Age (years)	53	77	28
Sex	Male	Male	Female
Race	Caucasian	Hispanic	Caucasian
Past medical history			
Diabetes mellitus	No	No	No
Hypertension	Recent onset (last 2 months)	Recent onset (last 3 months)	Recent onset (last month)
Smoking (mean cumulative intake: pack/year)	Yes (53)	Yes (59)	Yes (14)
Signs and symptoms			
Respiratory	No[a]	One episode of mild hemoptysis	No
Edema	Mild lower extremity	Mild lower extremity	Mild lower extremity
Hematuria	Yes (microscopic)	Yes (gross)	Yes (microscopic)
Labs			
Serum creatinine at biopsy µmol/L (mg/dL)	283 (3.2)	398 (4.5)	230 (2.6)
Proteinuria	Yes	Yes	Yes
Quantitative proteinuria (grams/24 h)	2.8	1	6.4
Serum albumin (g/dL)	3.6	3.6	3.1
Serum complement (C3, C4)	WNR	WNR	WNR
Serum paraprotein (SPEP and/or immunofixation)	Negative	Not performed	Negative
Serum cryoglobulin	Not performed	Negative	Not performed
Hepatitis panel (HBV and HCV)	Negative	Negative	Negative
Anti-GBM antibodies	ELISA: negative western blot: not performed	ELISA: negative western blot: positive	ELISA: negative western blot: negative
ANA	Negative	Weak (1: 40) ; speckled	Negative
ANCA	Negative	Negative	Negative
Anti-dsDNA	Not performed	Negative	Not performed
Urine culture	Not performed	Not performed	Negative

WNR, within normal range; GBM, glomerular basement membranes; ELISA, enzyme-linked immunoabsorbent assay; ANCA, anti-neutrophilic cytoplasmic antibodies; HBV, hepatitis B virus; HCV, hepatitis C virus; dsDNA, double-stranded DNA.
[a]An old X-ray revealed nodular appearance; at that time a bronchial biopsy was suboptimal and non-diagnostic.

Table 2. Pathologic findings

	Patient 1	Patient 2	Patient 3
Light microscopy			
# Glomeruli	21	42	22
Global sclerosis (#, %)	1 (5%)	16 (38%)	9 (40%)
Segmental sclerosis (#, %)	0 (0%)	0 (0%)	0 (0%)
Glomerular capillaries' double contours	Occasional	Occasional	Several
Endocapillary proliferation	No	No	No
Cellular crescents (%)[a]	1 (5%)	2 (8%)	1 (8%)
Fibrocellular and/or fibrous crescents (%)[a]	2 (10%)	3 (12%)	2 (16%)
Mesangial matrix expansion	Diffuse	Diffuse	Diffuse
Mesangial cellularity	Mild	No	Mild
Mesangial nodules	Yes	Yes	Yes
Mesangiolysis	Yes	Yes	Yes
Interstitial inflammation	Mild and focal[b]	Mild and focal[b]	Mild and focal[b]
Interstitial fibrosis/tubular atrophy	Moderate	Moderate	Moderate
Arterial sclerosis	Moderate to severe	Severe	Moderate to severe
Arteriolar sclerosis	Moderate to severe	Severe	Moderate to severe
Immunofluorescence findings			
Glomerular granular immune-type deposits	No	No	No
Glomerular linear deposits	IgG (4+), kappa (4+), lambda (4+)	IgG (4+)	IgG (3+), kappa (3+), lambda (3+)
IgG subtypes	IgG2: 4+	IgG1 (4+), IgG4 (3+), IgG2 (2+)	IgG4 (3+), IgG2 (2+), IgG1 (2+), IgG3 (1+)
Electron microscopy findings			
Electron-dense deposits	No	No	No
Foot process effacement	Focal and mild	Focal and mild	Focal and moderate
Subendothelial expansion by electron-lucent fluffy material	Yes	Yes	Yes
Cellular interposition and double contours	Yes	Yes	Yes

GBM, glomerular basement membrane; TBM, tubular basement membrane.
[a]Percentage of non-globally sclerosed glomeruli.
[b]The inflammation is largely confined to the foci of atrophy.

segmental irregularities, wrinkling and thickening. Occasional small cellular crescents were detected in all cases (range: 5–8% of non-sclerosed glomeruli) (Figure 1C) as well as occasional older fibrocellular or fibrous crescents (range: 10–16% of non-sclerosed glomeruli) (Figure 1D). The interstitium revealed moderate atrophy accompanied by focal mild mononuclear inflammation largely confined to the foci of atrophy. Prominent arterial and arteriolar sclerosis was detected in all biopsies.

Immunofluorescence microscopy evaluation was always negative for granular immune-type deposits (Table 2). In contrast, all samples were characterized by the presence of intense linear IgG staining of the GBM (IgG: Figure 2A versus albumin: Figure 2B). Less intense linear GBM staining for kappa and lambda light chains was present in two of the three samples while complement was negative in all biopsies. IgG subtyping revealed different results in all three patients; IgG1 was predominant in patient 1, IgG2 in patient 2 and IgG4 was the most prominent subtype in patient 3.

In addition to mesangial expansion (Figure 2C), ultrastructural evaluation revealed widespread signs of significant endothelial cell injury with expansion of the subendothelial space by fluffy electron-lucent material and signs of glomerular capillary wall remodeling (Figure 2D). Some degree of visceral epithelial cell foot process effacement was appreciated in all samples (Figure 2C and D).

In all three biopsies, the diagnoses of immune complex GN, amyloidosis, monoclonal immunoglobulin deposition disease, fibrillary GN and immunotactoid glomerulopathy could be excluded based on the negative immunofluorescence and electron microscopy findings.

Treatment and follow-up

Patient 1 was treated with cyclophosphamide and prednisone but without plasmapheresis and showed only modest improvement in serum creatinine [from 283 to 239 µmol/L (3.2–2.7 mg/dL)]. Approximately 1 year post-biopsy, the patient developed steroid-induced diabetes mellitus. Following the kidney biopsy, the patient quit smoking for a few months and resumed smoking later. About 18 months after the biopsy, the patient resumed smoking ∼10 cigarettes per day, had chronic kidney disease stage IV with a serum creatinine of 230 µmol/L (2.6 mg/dL).

Patient 2 was treated with prednisone, cyclophosphamide and plasmapheresis. Anti-GBM antibodies became undetectable by western blot and serum creatinine decreased from 398 to 265 µmol/L (4.5–3 mg/dL) 3 weeks after the beginning of treatment. However, 1 month later, the patient's renal function deteriorated again with a serum creatinine of 433 µmol/L (4.9 mg/dL) and he eventually became hemodialysis dependent.

Patient 3 was treated with SoluMedrol and was put on lisinopril. About 2 years after the biopsy, this patient had functioning kidneys but with an elevated serum creatinine of 194–212 µmol/L (2.2–2.4 mg/dL), 500 µg/24 h of proteinuria and no hematuria.

Discussion

Although nodular glomerulosclerosis is a characteristic finding in patients with diabetic nephropathy [10], it is not entirely specific for that entity. A similar pattern of injury can be observed in other conditions, such as immune complex-mediated GN (e.g. long-standing membranoproliferative GN), amyloidosis, monoclonal immunoglobulin deposition disease, and fibrillary and immunotactoid glomerulopathy, which can be ruled out by careful immunofluorescence and electron microscopy evaluation [5]. In 1999, the term ING was applied to describe nodular glomerulosclerosis occurring in the absence of all the

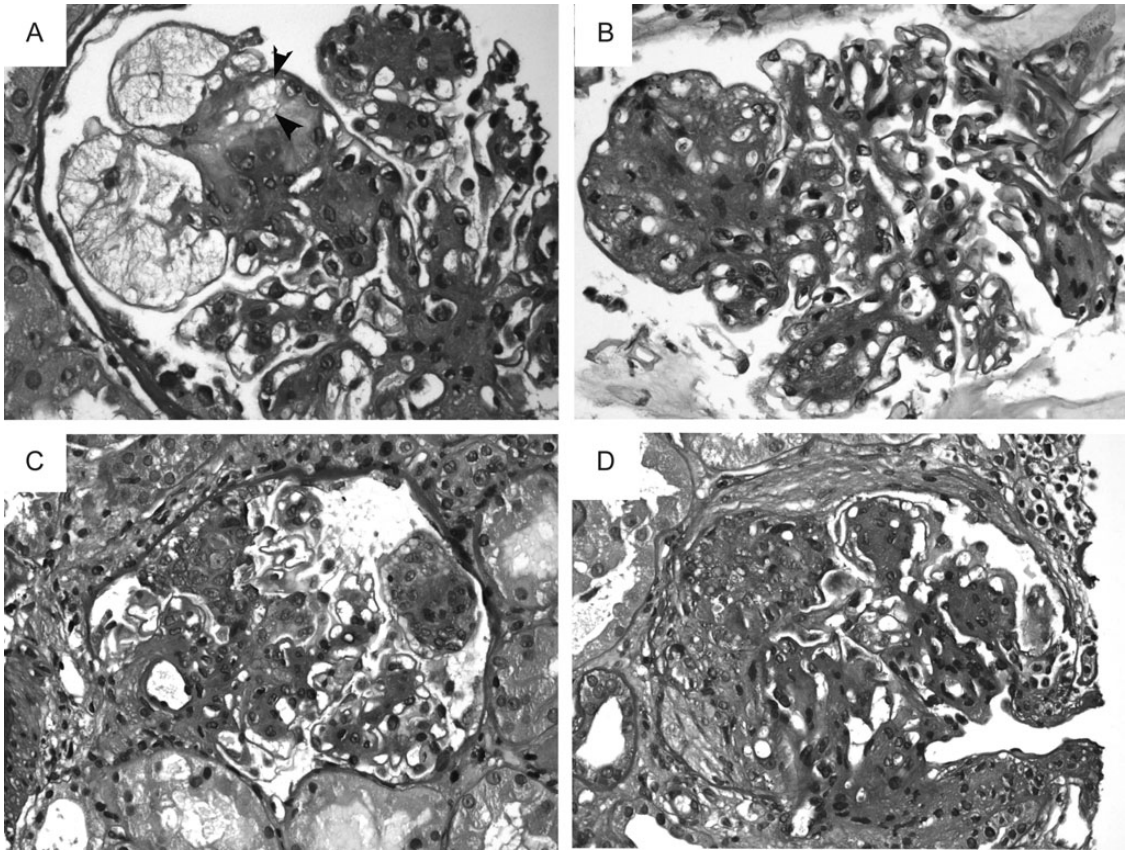

Fig. 1. (**A**) Glomerulus with nodular mesangial expansion and mesangiolysis (arrowheads); two capillary loops on the left show microaneurysmal dilatation and endothelial cell swelling (periodic acid-Schiff stain, original magnification ×600). (**B**) Prominent mesangial expansion due to segmental mesangiolysis is noted on the left side of the tuft; the remaining portion of the tuft shows mild mesangial expansion and no significant hypercellularity (periodic acid-Schiff stain, original magnification ×600). (**C**) Glomerulus with a small cellular crescent and mesangial expansion (periodic acid-Schiff stain, original magnification ×400). (**D**) Glomerulus with a healing extracapillary inflammatory lesion (fibrocellular crescent) and nodular mesangial expansion (periodic acid-Schiff stain, original magnification ×400).

aforementioned conditions [11]. A relatively large study by Markowitz *et al.* [3] revealed that ING is encountered in ∼0.5% of total renal biopsies and that the vast majority of patients with ING had a significant history of cigarette smoking (mean cumulative intake of 53 packs/year) and hypertension. These findings were soon confirmed by others [4]. In contrast to cigarette smoking, the causative relationship between hypertension and ING is less defined; (i) not infrequently, ING can be encountered in the absence of well-established hypertension [6] and (ii) hypertension could arguably be considered the result rather than the cause of renal endothelial cell injury [12]. In fact, smoking is known to induce direct oxidant injury to the endothelium and elevation of blood pressure [13, 14], which decreases again following smoking cessation [15]. Furthermore, ultrastructural signs of endothelial cell injury were appreciated in the majority of ING samples from chronic smokers who lacked a history of hypertension [6]. In the current study, all our patients developed a new onset of hypertension up to 3 months before biopsies and showed ultrastructural changes of widespread endothelial cell injury and significant glomerular capillary wall remodeling. These findings could argue that the hypertension observed in our patients appears to be the result of endothelial cell injury probably triggered or aggravated by cigarette smoking.

Anti-GBM disease is characterized by vascular injury mediated by autoimmune anti-GBM antibodies which could be initiated following a pathologic conformational change in the GBM structures [16]. Such antibodies are classically targeted against the α3 chain of type IV collagen [17, 18]. Anti-GBM-GN is the most aggressive form of GN [19] which often presents as a diffuse crescentic GN [20]. The diagnosis of anti-GBM GN typically requires the demonstration of serum anti-GBM antibodies together with the typical histologic manifestation of anti-GBM-GN including intense linear IgG staining along the GBM[20]. Detection of serum anti-GBM antibodies can be performed by ELISA or western blot techniques. The latter is more sensitive and specific and is particularly useful in patients with low antibody titers [21]. Importantly, some studies have shown that up to 13 and 3% of patients with anti-GBM-GN had undetectable antibodies by ELISA [22] or by both ELISA and western blot [20, 23], respectively. This could sometimes be attributed to the sensitivity of the currently used assays; using a highly sensitive IAsys biosensor assay, Salama *et al.* [23] confirmed the presence of anti-α3 chain of type IV collagen antibodies in both patients with crescentic GN and intense linear IgG staining along the GBM who had undetectable antibodies by both ELISA and western blot methods (100%). The absence of detectable antibodies could sometimes be ascribed to the fact that some cases might develop antibodies to other GBM antigens [24] possibly triggered by GBM injury and exposure of cryptic antigens; anti-GBM antibodies targeted against agrin were observed in renal allograft recipients

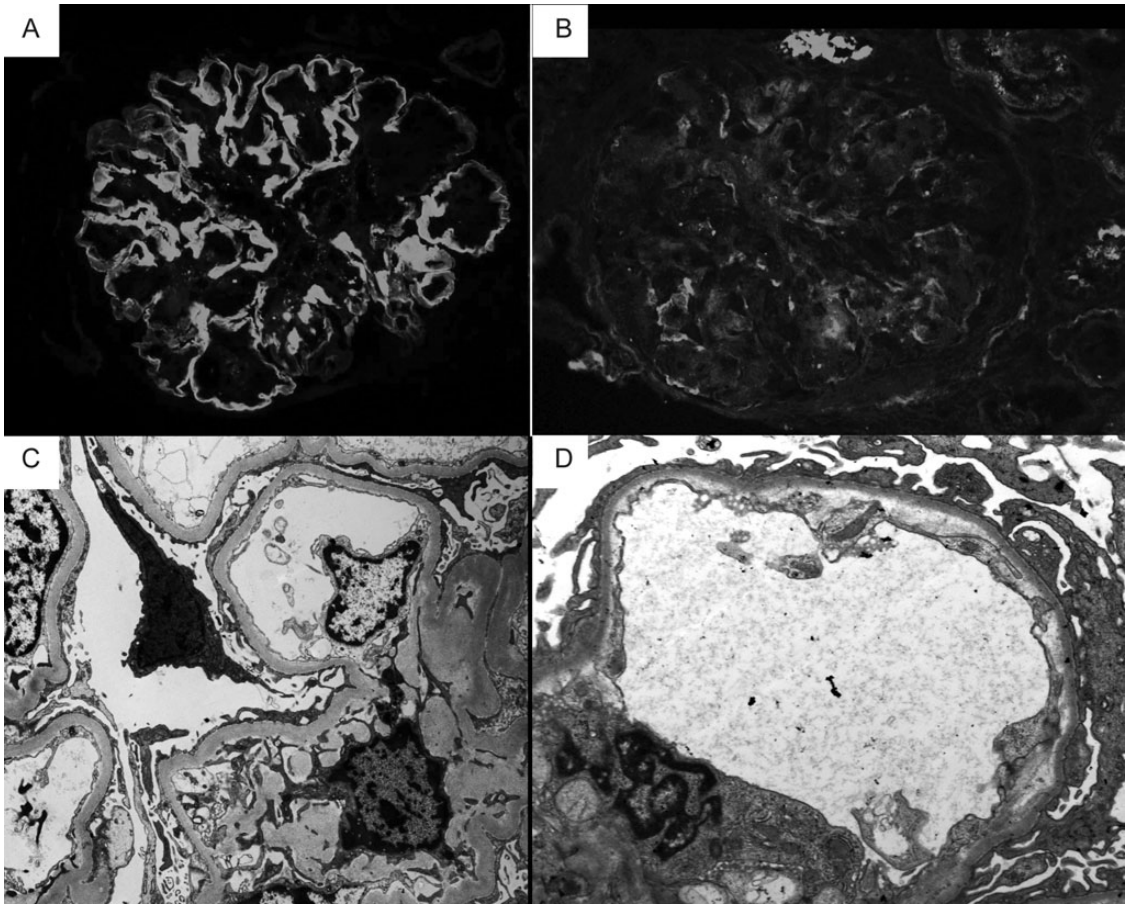

Fig. 2. (**A**) Immunofluorescence evaluation revealed an intense linear staining for immunoglobulin G (IgG antibody, immunofluorescence microscopy, original magnification ×200). (**B**) Albumin staining of the same glomerulus in picture (**A**) revealed no significant linear reactivity along the glomerular or tubular basement membranes (immunofluorescence microscopy, original magnification ×400). (**C**) Electron microscopy examination revealed mesangial expansion by matrix and mild effacement of podocyte foot processes, in the absence of electron-dense deposits (electron microscopy, original magnification ×3000). (**D**) Ultrastructural detail of a peripheral capillary wall with subendothelial expansion by electron-lucent fluffy material; discrete wisps of new basement membrane material are formed under the displaced endothelium (electron microscopy, original magnification ×8000).

with chronic transplant glomerulopathy who had previous episodes of rejection [25]. These aforementioned data have prompted some authorities to consider histologic findings to be diagnostic for anti-GBM disease if compatible with clinical features despite negative antibody detection in the serum [23].

A causative relationship between cigarette smoking and anti-GBM disease has been suggested based on the high prevalence of cigarette smoking in patients with Goodpasture syndrome and pulmonary manifestations [7–9]. Such association was less obvious in patients with isolated anti-GBM-GN [26–28]. It has been proposed that smoking can increase endothelial permeability and enable preexisting antibodies to bind to their basement membrane antigens [9] or can induce direct basement membrane injury [9] and inhibit the enzymes which catalyze formation of sulfilimine bonds [16] to expose cryptic antigens and promote antibody formation. All our biopsies were associated with ultrastructural signs of endothelial cell injury and glomerular capillary remodeling which could have contributed to antigen exposure and/or antibody binding.

Despite the association of smoking with each of ING an anti-GBM disease, no reports of anti-GBM-GN in the presence of ING on a kidney biopsy have been previously published. Interestingly, however, anti-GBM antibodies were detectable in 9% of patients with IGN in the study by Markowitz *et al.* [3] but without any description of crescents. In the current study, we describe the first case series of patients with non-diabetic nodular glomerulosclerosis and anti-GBM-like GN. All our patients were heavy smokers. Although all these cases were encountered in the last 2 years, a search for a combination of nodular glomerulosclerosis and features of anti-GBM GN during the past 7 years in our archives failed to uncover additional cases, suggesting that this pattern of glomerular injury is rarely encountered.

Kidney biopsy from all three patients revealed ultrastructural signs of endothelial injury, diffuse mesangial matrix expansion, nodular pattern of glomerulosclerosis, intense linear IgG staining along the GBM in the presence of occasional cellular, fibrocellular and fibrous crescents. In our study, the superimposed anti-GBM-like GN was atypical for the following reasons: (i) anti-GBM antibodies in serum were only detectable in one of three (33%) patients. We cannot ascertain whether the lack of detectable anti-GBM antibodies in the other two patients was due to low titer and the lack of sensitivity (western blot testing was not performed in patient 1), or to the presence of antibodies targeted to different GBM antigens. (ii) In contrast to classic anti-GBM-GN where IgG1 is the predominant subtype [8], IgG2 and IgG4 subtypes predominate in 2 of 3 of our patients. IgG2 fixes complement poorly

while IgG4 is not capable of fixing the complement [29, 30]. In anti-GBM-GN, these subtypes of antibodies are rarely encountered and often associated with a less aggressive form of the disease [31]. (iii) In our series, occasional non-circumferential crescents in different stages of development were always observed; this is different from typical anti-GBM-GN where the biopsy usually reveals synchronized and circumferential crescents involving the majority of glomeruli [22].

Aggressive treatment with plasmapheresis was performed only in one patient who lost his kidney function, while the other two patients who were treated less aggressively have been dialysis-free, at least till the time of writing. Of note, the patient who lost the kidney function had prominent endothelial cell injury and significant chronic damage on the kidney biopsy, and he was the only one with detectable anti-GBM antibodies in serum by western blot, with a predominance of IgG1 subtype on immunofluorescence microscopy.

In conclusion, we report here the first case series of a rare but distinct pattern of glomerular injury observed in heavy smokers without clinical history of diabetes, with kidney biopsy findings of nodular glomerulosclerosis, endothelial cell injury, linear anti-GBM effect on immunofluorescence studies, and occasional crescent formation in some glomeruli. Our findings highlight the important association of smoking with endothelial injury, glomerular mesangial expansion and crescent formation. Our study also highlights the need for a prudent search for cellular crescents when strong intense linear IgG is observed along the GBM. Finally, the fact that this pattern of dual injury is not observed in all patients who smoke heavily suggests that a possible second hit or a genetic predisposition may be necessary to develop such complication.

Conflict of interest statement. None declared.

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